Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer

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Yang, Heping, Zong-Zhi Huang, Zhaohui Zeng, Changjin Chen, Robert R. Selby, and Shelly C. Lu. Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer. Am J Physiol Gastrointest Liver Physiol 280: G184–G190, 2001.—Methionine adenosyltransferase (MAT), an essential enzyme that catalyzes the formation of S-adenosylmethionine (SAM), is encoded by two genes, MAT1A (liver-specific) and MAT2A (non-liver-specific). We showed a switch from MAT1A to MAT2A expression in human liver cancer, which facilitates cancer cell growth. The present work examined the role of methylation in MAT2A transcriptional regulation. We found that the human MAT2A promoter is hypomethylated in hepato-cellular carcinoma, in which the gene is upregulated transcriptionally, but hypermethylated in normal liver, in which the gene is minimally expressed. Luciferase activities driven by in vitro methylated MAT2A promoter constructs were 75–95% lower than activities driven by unmethylated constructs. SAM treatment of Hep G2 cells reduced MAT2A endogenous expression by 75%, hypermethylated the MAT2A promoter, and reduced luciferase activities driven by MAT2A promoter constructs by 65–75% while not affecting MAT1A’s promoter activity. Treatment of adult rat and human hepatocytes with trichostatin A, an inhibitor of histone deacetylase, upregulated MAT2A expression by more than fourfold. Collectively, these results suggest that MAT2A expression is regulated by promoter methylation and histone acetylation.

Methionine adenosyltransferase (MAT) is a critical cellular enzyme that catalyzes the formation of S-adenosylmethionine (SAM), the principal biological methyl donor and the ultimate source of the propylamine moiety used in polyamine biosynthesis (9, 23). In mammals, two different genes, MAT1A and MAT2A, encode for two homologous MAT catalytic subunits, α1 and α2 (1, 13, 17). MAT1A is expressed only in liver, and it encodes the α1 subunit found in two native MAT isoforms, which are either a dimer (MAT III) or a tetramer (MAT I) of this single subunit (17). MAT2A encodes for a catalytic subunit (α2) found in a native MAT isozyme (MAT II), which is associated with a catalytically inactive regulatory subunit (β) in lymphocytes encoded by yet a third gene (8, 17). MAT2A is widely distributed (1, 13, 17). MAT2A also predominates in the fetal liver and is progressively replaced by MAT1A during liver development (11, 12). In adult liver, increased expression of MAT2A is associated with rapid growth or dedifferentiation of the liver.

We showed a switch in the gene expression from MAT1A to MAT2A in human liver cancer (6) from 12 to 24 h after partial hepatectomy in the rat (14) and after treatment with thioacetamide (15). Using a cell line model that differs only in the type of MAT expressed, we demonstrated that the type of MAT expressed by the cell significantly influences the rate of cell growth (5). Specifically, MAT1A expression is associated with the lowest rate of cell growth, whereas the opposite is true of MAT2A expression (5). Thus the switch in MAT expression in liver cancer may play an important pathogenetic role by facilitating liver cancer growth. Little is known about transcriptional regulation of MAT2A. To understand better the mechanism for transcriptional regulation of human MAT2A, we cloned the human MAT2A promoter (22). The MAT2A promoter contains numerous CpG dinucleotides (22), and in all the conditions in which the hepatic expression of MAT2A is increased, DNA is hypomethylated globally (5, 6, 14, 15). In the present work, we examined directly whether methylation status of the human MAT2A promoter plays a role in the regulation of MAT2A expression.

EXPERIMENTAL PROCEDURES

Materials. Trichostatin A was obtained from Sigma Chemical (St. Louis, MO). SAM was a kind gift from Dr. Pablo Ortiz (Boehringer Ingelheim, Madrid, Spain). Cell culture media and fetal bovine serum were obtained from Gibco BRL Life Technologies (Grand Island, NY). The luciferase assay system and the β-galactosidase enzyme assay system were obtained from Promega (Madison, WI). All restriction enzymes were obtained from either Promega or Gibco. Sss

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I methylase was obtained from New England BioLabs (Beverly, MA). \(^{32}P\)dCTP (3,000 Ci/mmol) and \(^{32}P\)dUTP (3,000 Ci/mmol) were purchased from NEN (Boston, MA). All other reagents were of analytical grade and were obtained from commercial sources.

*Source of normal and cancerous liver tissue.* Normal liver tissue was obtained from normal liver included in the resected liver specimens of five patients with metastatic colon or breast carcinoma. Cancerous liver tissues were obtained from five patients undergoing surgical resection for primary hepatocellular carcinoma (HCC). Written informed consent was obtained from each patient. The contamination of HCC specimens, rat and human hepatocytes, and Hep G2 cells according to the method of Chomczynski and Sacchi (7) was determined using a hemocytometer. These tissues were immediately frozen in liquid nitrogen for subsequent isolation of RNA, DNA, nuclei, and cytosols.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the University of Southern California School of Medicine’s human research review committee.

Procedures and human liver specimen culture. Primary cultures of rat hepatocytes were provided by the Cell Culture Core of the USC Liver Disease Research Center. Isolation and culture of rat hepatocytes were done as described previously (6).

Primary cultures of human hepatocytes were prepared by the Cell Culture Core of the USC Liver Disease Research Center as described previously (6) from normal tissue included in the resected liver specimens of patients with metastatic colon carcinoma to the liver.

To study the effect of trichostatin A on MAT2A expression, the agent was added at the time of medium change, 4 h after plating of hepatocytes. RNA was obtained from vehicle- and trichostatin A-treated cells 18 h later.

*Nucleic acid extraction.* RNA was isolated from frozen liver specimens, rat and human hepatocytes, and Hep G2 cells according to the method of Chomczynski and Sacchi (7). Poly(A) RNA (mRNA) was obtained using oligo(dT) cellulose columns according to the protocol provided by Life Technologies (no. 15939–010). Genomic DNA was isolated from frozen human liver specimens and Hep G2 cells according to standard procedures (28) and used for Southern blot analysis (see Restriction enzyme digestion and Southern blot analysis).

_Northern blot analysis._ Northern blot analysis was performed on total RNA or poly(A) RNA using the specific MAT2A cDNA probe as described previously (6). To ensure equal loading of RNA samples and transfer in each of the lanes, the same membranes were also rehybridized with \(^{32}P\)-labeled 18S cDNA or \(\beta\)-actin probes as described previously (4, 14). To ensure that the effect of trichostatin A was specific, the same membranes were also hybridized with a specific cDNA probe for the heavy subunit of \(\gamma\)-glutamylcysteine synthetase (4), which is not known to be regulated by methylation (20). All cDNA probes were labeled with \(^{32}P\)CTP using a random primer kit (Primer-It II Kit; Stratagene, La Jolla, CA). Autoradiography and densitometry (Gel Documentation System, Scientific Technologies, Carlsbad, CA; NIH Image 1.60 software program) were used to quantify relative RNA. Results of Northern blot analysis were normalized to 18S cDNA or \(\beta\)-actin.

_Western blot analysis._ The steady-state protein levels of non-liver-specific MAT in normal liver and HCC were determined by Western blot analysis as described previously (15).

_Nuclear run-on transcription assay._ Isolation of nuclei and nuclear run-on transcription assay were done as described previously (6, 14). Briefly, \(2 \times 10^7\) nuclei (determined using a hemocytometer) were resuspended in 50 \(\mu\)l of nuclei-freezing buffer (50 mM Tris-HCl, pH 8.0, 50% [vol/vol] glycerol, 5 mM MgCl\(_2\), 0.1 mM EDTA), snap-frozen with liquid N\(_2\), and kept at \(-80^\circ\)C. After nuclear run-on assay with isolated nuclei, identical amounts of labeled nuclear RNAs (1 \(\times 10^8\) cpm) were hybridized overnight with MAT2A or \(\beta\)-actin cDNAs (2 \(\mu\)g each) with a slot blot apparatus (Bio-Dot SF, Bio-Rad, Hercules, CA) as described previously (6, 14). After hybridization, filters were washed and subjected to autoradiography and densitometry as described in Northern blot analysis. Results of nuclear run-on assay were normalized to \(\beta\)-actin.

_Restriction enzyme digestion and Southern blot analysis._ Msp I and Hpa II restriction endonucleases were used as described previously with modifications (10). Msp I and Hpa II can distinguish between the unmethylated and methylated cytosine in the nucleotide sequence 5’-CCGG. Msp I is insensitive to methylation status, whereas Hpa II will digest only if the internal cytosine is unmethylated (10). Genomic DNA samples (15 \(\mu\)g each) were digested with 45 units of enzymes for 6 h at 37°C. The digested DNA was separated on 1% agarose, stained with ethidium bromide to allow assessment of completeness of digestion (would produce a smear along the entire lane), transferred onto nylon filters by 20\(\times\) SSC (sodium saline citrate), and crosslinked in an ultraviolet crosslinker (FS-UVXL-1000, Fisher Biotech). The filters were prehybridized at 65°C in Rapid-hybridization buffer (Amer sham Life Sciences) for 20 min and hybridized with a \(^{32}P\)labeled 1329/60 fragment of the human MAT2A promoter (22) for 3 h at 65°C in the same buffer. The blots were washed twice at room temperature for 20 min in 2\(\times\) SSC containing 0.1% SDS and at 65°C for 30 min in 0.1\(\times\) SSC containing 0.1% SDS. Autoradiography was performed by exposure to Kodak BioMax MR film at \(-80^\circ\)C.

_In vitro methylation reactions._ To examine region-specific methylation, MAT2A promoter fragments were obtained by digesting the MAT2A promoter-luciferase (Luc) constructs with appropriate restriction enzymes as described previously (22). MAT2A promoter fragments were methylated using Sss I methylase following the manufacturer’s protocol. In parallel control reactions, the same amount of promoter fragments were subjected to mock methylation (incubated with methylase in the absence of SAM). The extent of methylation after each reaction was determined by digestion with Hpa II. Methylated and mock-methylated MAT2A promoter fragments were religated into the promoterless luciferase pGL-3 enhancer vector from which they were excised. To make sure only the religated plasmids were used for transfection, the religated products were separated on a 1% agarose gel and compared with MAT2A promoter fragments alone, MAT2A promoter-Luc constructs, or empty pGL-3 enhancer plasmid. For each construct, the position of the religated product was exactly the same as the control MAT2A promoter-Luc construct and different from MAT2A fragment alone or empty plasmid. The band that corresponded to the MAT2A promoter-Luc construct was cut out and used for subsequent transfection. To control for the amount of DNA used in transfection, DNA concentrations were measured spectrophotometrically and DNA samples were separated on a 1% agarose gel. Densitometric analysis was performed to ensure that the amount of DNA used was the same from each construct.

_Transfection assays._ Hep G2 cells were obtained from American Type Culture Collection (Manassas, VA) and cultured according to the instructions provided. To study the effect of methylation on relative transcriptional activities of the MAT2A promoter fragments, Hep G2 cells (1 \(\times 10^6\) cells in 4 ml of medium) were transiently transfected with 8 \(\mu\)g of unmethylated or methylated MAT2A promoter-luciferase...
gene construct or promoterless pGL3-enhancer vector (as negative control) and 2 μg of a β-galactosidase expression plasmid (as an internal standard of transfection efficiency) using the calcium phosphate precipitation method (28). After 24 h, cells were harvested and lysed in 1 ml of reporter lysis buffer (luciferase assay system; Promega). The luciferase assay was performed on 20 μl of the cleared lysate and 100 μl of luciferase assay reagent using a TD-20/20 luminometer (Promega). The β-galactosidase assay was done according to the supplier’s instructions (β-galactosidase enzyme assay system; Promega) using 150 μl of the cell lysate. The luciferase activity of each transfection was expressed as luciferase activity/β-galactosidase activity.

Effect of SAM on MAT promoter activity, endogenous MAT2A expression, and methylation. We (5) previously showed that treatment of liver cancer cells with SAM in the culture medium increased the levels of intracellular SAM and global DNA methylation. To examine the effect of SAM on MAT promoter activity, SAM (0.5 mM) or vehicle was added to culture medium during the last 20 h of transfection with the human MAT2A (22) or MAT1A (31) promoter-Luc constructs. Luciferase activity was measured as described in Transfection assays. To see the effect of SAM on endogenous MAT2A expression, RNA was extracted from Hep G2 cells treated with SAM (0.5 mM) or vehicle for 20 h, and the expression of MAT2A was determined by Northern blot analysis as described previously (6). To examine the effect of SAM on methylation pattern, DNA was extracted from Hep G2 cells treated with SAM (0.5 mM) or vehicle for 20 h, subjected to restriction enzyme digestion with Msp I, Hpa II, and processed for Southern blot analysis using the human MAT2A promoter as probe as described in Restriction enzyme digestion and Southern blot analysis.

Statistical analysis. Data are given as means ± SE. Statistical analysis was performed using ANOVA followed by Fisher’s test for multiple comparisons and paired Student’s t-test for paired analysis. Significance was defined as P < 0.05.

![Fig. 1](image1.png) **Fig. 1.** Comparison of steady-state MAT2A mRNA (A) and non-liver-specific methionine adenosyltransferase (MAT) protein (B) levels in normal liver and hepatocellular carcinoma (HCC). A: Poly(A) RNA (3 μg each lane) samples obtained from 5 normal liver and 5 HCC samples were analyzed by Northern blot hybridization with a 32P-labeled MAT2A cDNA probe as described in EXPERIMENTAL PROCEDURES. The same membrane was then rehybridized with 32P-labeled 18S cDNA probe. B: liver cytosol (50 μg/lane) obtained from 3 normal liver and 3 HCC samples was analyzed by Western blot analysis using anti-non-liver-specific MAT antibodies as described in EXPERIMENTAL PROCEDURES. Equivalent protein loading was ensured by Coomassie blue staining of gels after transblotting (not shown).

![Fig. 2](image2.png) **Fig. 2.** MAT2A gene transcription in normal liver and HCC. Nuclei were isolated from normal liver and HCC for nuclear run-on transcription assay performed as described in EXPERIMENTAL PROCEDURES. Identical amounts of labeled nuclear RNA from both normal liver and HCC were hybridized with MAT2A or β-actin probes.

**RESULTS**

Expression of MAT2A in human liver cancer. Figure 1 shows that the steady-state MAT2A mRNA level is markedly increased in HCC (600% of normal) and non-liver-specific MAT protein level is similarly increased. Nuclear run-on assay showed that the molecular mechanism for this increase is largely increased transcription (450% of normal) (Fig. 2).

**MAT2A methylation in normal and cancerous liver.** To see whether there is any difference in the methylation pattern of the human MAT2A promoter in normal versus cancerous liver, Southern blot analysis was performed after treating liver DNA with Msp I or Hpa II restriction endonucleases and hybridizing with the labeled cloned human MAT2A promoter (22). There are 15 Msp I/Hpa II restriction sites in the 5′-flanking region (−1329 to +64, where +1 is the transcriptional start site) of MAT2A (−12, −43, −131, −176, −351, −365, −455, −470, −507, −551, −562, −568, −636, −1212, and −1314). The largest fragment is 576 bp, followed by 175 and 102 bp; the flanking fragments are 76 and 15 bp. As shown in Fig. 3, digestion of DNA from normal or cancerous liver with Msp I, which is insensitive to methylation of the internal cytosine residue of CCGG sequences, resulted in the same number

![Fig. 3](image3.png) **Fig. 3.** MAT2A promoter methylation pattern in normal liver and HCC. DNA from 5 normal liver and 5 HCC samples were digested with Msp I, which is insensitive to methylation of the internal cytosine residue of CCGG sequences, or Hpa II, which is inhibited if the internal cytosine is methylated, and Southern blot hybridization was performed using the labeled human MAT2A promoter as described in EXPERIMENTAL PROCEDURES. Note that digestion of normal liver or HCC DNA with Msp I resulted in the same number and size of bands. However, digestion of DNA with Hpa II resulted in high-molecular-weight bands only in normal liver (arrow on right) but multiple lower-molecular-weight bands only in HCC (arrow on left).
and size of bands. However, digestion of DNA with Hpa II, which is inhibited if the internal cytosine is methylated, resulted in high-molecular-weight bands only in normal liver but lower-molecular-weight bands only in HCC (Fig. 3).

Effect of methylation on MAT2A promoter activity. To better establish a causal role of MAT2A promoter methylation on promoter activity, promoter fragments obtained by digestion of the MAT2A promoter-Luc constructs with appropriate restriction enzymes were methylated using Sss I methylase or subjected to mock methylation. Figure 4 shows the effectiveness of in vitro methylation, because Hpa II was able to digest only mock-methylated MAT2A promoter fragments.

Transfection of Hep G2 cells with mock-methylated MAT2A promoter constructs showed several regions in the promoter to be important for overall promoter activity (Fig. 5). The construct −47/+60 increased luciferase activity by 96-fold over vector control. This region contains the TATA box and produced 4% of the maximal activity. The construct −270/+60 increased luciferase activity by ~1,000-fold or 46% of the maximal activity. The construct −571/+60 produced the maximal promoter activity. Inclusion of additional upstream sequences (−939/+60 or −1329/+60) resulted in luciferase activities not significantly different from the −571/+60 construct.

The effect of methylation on MAT2A promoter activity is shown in Fig. 5. Transfection of Hep G2 cells with in vitro methylated MAT2A promoter-Luc constructs resulted in 75–95% lower luciferase activity compared with the activity driven by mock-methylated MAT2A promoter constructs.

Effect of SAM treatment on MAT promoter activity. To see whether increased methylation in vivo can also alter MAT2A promoter activity, Hep G2 cells were transfected with unmethylated MAT2A promoter constructs and treated with SAM (0.5 mM) in culture medium. Figure 5 shows that, similar to in vitro methylation of MAT2A promoter constructs, SAM treatment of Hep G2 cells transfected with unmethylated MAT2A promoter constructs also inhibited MAT2A promoter activity. The degree of inhibition by SAM treatment was 65–75% for the MAT2A promoter-Luc constructs.

To see whether the effect of SAM on MAT2A promoter activity is promoter specific, Hep G2 cells were transfected with MAT1A promoter constructs (31) and treated with SAM at the same dose and duration. This had no effect on luciferase activity driven by the human MAT1A promoter (Fig. 6).

Effect of SAM treatment on endogenous MAT2A expression and methylation. We next examined whether SAM treatment altered the endogenous expression of MAT2A in Hep G2 cells and whether this correlated with a change in the methylation status of the promoter. Figure 7A shows that SAM treatment led to a significant reduction of the steady-state MAT2A mRNA level (25 ± 2% of control by densitometric analysis, result represents mean ± SE from 3 separate determinations; *P < 0.05 vs. the pGL-3 enhancer control, its respective methylated construct, and SAM treatment; ††P < 0.05 vs. the pGL-3 control, −47/+60-Luc, its respective methylated construct, and SAM treatment; †P < 0.05 vs. pGL-3 control, −47/+60-Luc, −270/+60-Luc, respective methylated constructs, and SAM treatment (ANOVA followed by Fisher’s test).
Effect of trichostatin A treatment on MAT2A expression in normal hepatocytes. Recently, the mechanism of methylation-dependent gene silencing has been shown to involve binding of histone deacetylase and methylcytosine-binding protein such as MeCP2, which appear to be essential for the assembly of the methylated sequences into a condensed state (2, 16). The silenced methylated gene can be relieved by the deacetylase inhibitor trichostatin A (24, 27). To see whether low expression of MAT2A in normal adult hepatocytes is caused by this mechanism, normal rat and human hepatocytes in primary culture were treated with trichostatin A for 18 h. Figure 8 shows that treatment of both rat and human hepatocytes with the deacetylase inhibitor led to increased expression of MAT2A by nearly fivefold. Specificity of the trichostatin A effect is also confirmed, because it exerted no influence on the steady-state mRNA level of the heavy subunit of \( \gamma \)-glutamylcysteine synthetase, which is not known to be modulated by methylation (20).

DISCUSSION

In mammals, two distinct genes encode for the enzyme MAT (1, 13, 17). MAT1A is a liver-specific gene that is expressed in the liver shortly before birth and becomes the major form of MAT as the liver matures (11, 12). It is a marker for the differentiated or mature liver phenotype. In contrast, MAT2A is expressed in all nonhepatic tissues as well as during periods of rapid liver growth (5, 6, 8, 11–15). Although the two MAT genes are highly homologous, the enzymes they encode for are different in kinetic profiles and regulatory properties (19). The kinetic parameters varied in different studies depending on the purification procedure and purity of the enzyme. The Michaelis constant for methionine is lowest for non-liver-specific MAT or MAT II (\( < 10 \) \( \mu \)M), intermediate for MAT I (23 \( \mu \)M–1 mM), and highest for MAT III (215 \( \mu \)M–7 mM), with different studies reporting different absolute values (3, 18, 25, 26, 29). The activity of MAT is also modulated by
SAM, the product of the reaction it catalyzes. SAM strongly inhibits MAT II ($IC_{50} = 60 \mu M$), whereas it minimally inhibits MAT I ($IC_{50} = 400 \mu M$) and stimulates MAT III (up to 8-fold at 500 $\mu M$ SAM concentration) (29). Because of these differences, the type of MAT expressed by a cell can influence the cell’s steady-state SAM level and methylation status. Consistent with this, in a cell line model that differs only in the type of MAT expressed, cells that expressed MAT1A had the highest intracellular SAM level and DNA methylation whereas cells that expressed MAT2A had the lowest (5). Interestingly, cells that expressed MAT2A grew faster than cells that expressed MAT1A (5). Thus the switch in MAT expression in liver cancer may be pathogenetically important because it offers the cancerous cell a more rapid growth rate.

Despite the importance of MAT2A, little is known about its gene regulation. MAT2A gene expression appears to be influenced by the cell cycle, as evident by its induction during liver regeneration and T lymphocyte activation (14, 30). In both cases, the mechanism involved in part increased transcription (14, 30). It has been speculated that the induction in MAT2A and MAT II may be a mechanism for the cell to provide an increased supply of SAM, the precursor to polyamine synthesis that is required for cell growth (30). Our laboratory has been interested in studying regulation of MAT2A expression in liver. We have described four conditions in which MAT2A expression is induced, namely, liver cancer, during liver regeneration, after thioacetamide treatment, and in alcoholic liver injury (6, 14, 15, 21). All four conditions have in common global DNA hypomethylation. The cloned human MAT2A promoter contains numerous CpG dinucleotides (22), which led us to speculate that MAT2A expression may be regulated by methylation.

Using resected HCC samples, we confirmed our previous finding that the steady-state MAT2A expression is greatly induced (6). For the first time, we showed that increased transcription accounted for a large part of the MAT2A gene induction. When MAT2A promoter methylation status was examined, we found that MAT2A promoter is hypomethylated in HCC, in which the gene is upregulated transcriptionally, but hypermethylated in normal liver, in which the gene is minimally expressed. The significance of this finding was confirmed using in vitro methylated MAT2A promoter constructs that were repressed when transfected in Hep G2 cells.

Because transfection of in vitro methylated promoter constructs may not represent a physiologically relevant event, we next evaluated in vivo methylation of MAT2A promoter. This was accomplished by treating Hep G2 cells with SAM, which we (5) had shown previously to increase cellular SAM level and global DNA methylation. SAM treatment of Hep G2 cells transfected with unmethylated MAT2A promoter constructs also inhibited the MAT2A promoter activity, further confirming the importance of methylation in its transcriptional regulation. The specificity of SAM’s effect is confirmed because it had no influence on the luciferase activity driven by the human MAT1A promoter.

We further established a possible regulatory effect of promoter methylation on MAT2A gene expression by showing that SAM treatment of Hep G2 cells decreased the expression of endogenous MAT2A and methylated its promoter. The mechanism of how this occurs is unclear at the present. One possibility is induction of the expression and/or activity of DNA methyltransferase(s), which then methylates the MAT2A promoter. This subject requires further investigation.

Although CpG methylation in mammalian DNA has been linked for many years to gene silencing and an inactive chromatin structure, the mechanism of how this is achieved is only being revealed in recent years (27). Studies by Jones et al. (16) and Nan et al. (24) showed that recruitment of histone deacetylases to methylated 5’-CpG dinucleotides through methylcytosine-binding proteins such as MeCP2 can lead to a condensed and inactive chromatin. Both groups also showed that inhibition of histone deacetylase by trichostatin A can overcome the methylation-dependent transcriptional silencing (16, 24). Thus transcriptional repression associated with cytosine methylation depends on histone deacetylation. To see whether histone deacetylation is also involved in the regulation of MAT2A expression, we treated normal rat and human hepatocytes with trichostatin A overnight. Marked increase in the MAT2A mRNA level after trichostatin A treatment suggests that this mechanism also regulates the gene expression of both human and rat MAT2A.

In summary, we have shown for the first time that human MAT2A is regulated by cytosine methylation.
Our data also suggest that SAM level itself may modulate MAT2A expression via promoter methylation.

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